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Genome-wide biochemical analysis of Arabidopsis protein phosphatase using a wheat cell-free system

Hirotaka Takahashi [a,b,1], Akihiko Ozawa [a,b,2], Keiichirou Nemoto [a,b], Akira Nozawa [a], Motoaki Seki [c], Kazuo Shinozaki [d], Hiroyuki Takeda [a,b], Yaeta Endo [a,b,e,f], Tatsuya Sawasaki [a,b,e,f,*]

[a] Cell-Free Science and Technology Research Center, Ehime University, Matsuyama 790-8577, Japan
[b] The Venture Business Laboratory, Ehime University, Matsuyama 790-8577, Japan
[c] Plant Genomic Network Research Team, RIKEN Plant Science Center, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan
[d] Gene Discovery Research Group, RIKEN Plant Science Center, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan
[e] Proteo-Medicine Research Center, Ehime University, Toon, Ehime 791-0295, Japan
[f] RIKEN Systems and Structural Biology Center, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan

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ABSTRACT

Plant genome possesses over 100 protein phosphatase (PPase) genes that are key regulators of signal transduction via phosphorylation/dephosphorylation event. Here we report a comprehensive functional analysis of protein serine/threonine, dual-specificity and tyrosine phosphatases using recombinant PPases produced by wheat cell-free protein synthesis system. Eighty-two recombinant PPases were successfully produced using Arabidopsis full-length cDNA as templates. In vitro PPase assay was performed using phosphorylated myelin basic protein as substrate. Among the AtPPases examined, 26 serine/threonine, three dual-specificity and one tyrosine PPases exhibited catalytic activity, including 20 serine/threonine and one dual-specificity PPases that showed in vitro activities for the first time. Our study demonstrates genome-wide biochemical analysis of AtPPases using wheat cell-free system, and provides new information and insights on enzyme activities.

Structured summary of protein interactions:
PTP1 dephosphorylates **MBP** by phosphatase assay (View interaction).
AtPP2C dephosphorylates **MBP** by phosphatase assay (View interaction).
POLTE dephosphorylates **MBP** by phosphatase assay (View interaction).
TOPP8 dephosphorylates **MBP** by phosphatase assay (View interaction).
HAB1 dephosphorylates **MBP** by phosphatase assay (View interaction).
ABI2 dephosphorylates **MBP** by phosphatase assay (View interaction).
At1g34750 dephosphorylates **MBP** by phosphatase assay (View interaction).
At1g43900 dephosphorylates **MBP** by phosphatase assay (View interaction).
At3g15260 dephosphorylates **MBP** by phosphatase assay (View interaction).
At5g53140 dephosphorylates **MBP** by phosphatase assay (View interaction).
At1g18030 dephosphorylates **MBP** by phosphatase assay (View interaction).
At3g06270 dephosphorylates **MBP** by phosphatase assay (View interaction).
At2g25070 dephosphorylates **MBP** by phosphatase assay (View interaction).
At3g02750 dephosphorylates **MBP** by phosphatase assay (View interaction).
At5g10740 dephosphorylates **MBP** by phosphatase assay (View interaction).
at4g26080 dephosphorylates **MBP** by phosphatase assay (View interaction).
At4g28400 dephosphorylates **MBP** by phosphatase assay (View interaction).

Abbreviations: PPase, protein phosphatase; Tyr, tyrosine; Ser, serine; Thr, threonine; PPPs, serine/threonine-specific protein phosphatases; PPMs, metal ion-dependent phosphoprotein phosphatases; PTPs, tyrosine-specific phosphatases; DSPs, dual-specificity phosphatases; AtPPases, Arabidopsis phosphoprotein phosphatases; MBP, myelin basic protein; bls, biotin ligation site; TCA, trichloroacetic acid; TLC, thin-layer chromatography

* Corresponding author. Address: Cell-Free Science and Technology Research Center, Ehime University, Bunkyo-cho, 3-ban, Matsuyama 790-8577, Japan. Fax: +81 89 927 9941.
E-mail address: sawasaki@ehime-u.ac.jp (T. Sawasaki).
[1] Present address: Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore.
[2] Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port St. Lucie, FL 34987, United States.

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At5g06750 dephosphorylates **MBP** by phosphatase assay (View interaction).
At4g31860 dephosphorylates **MBP** by phosphatase assay (View interaction).
At3g17250 dephosphorylates **MBP** by phosphatase assay (View interaction).
At4g38520 dephosphorylates **MBP** by phosphatase assay (View interaction).
At3g05640 dephosphorylates **MBP** by phosphatase assay (View interaction).
At5g66080 dephosphorylates **MBP** by phosphatase assay (View interaction).
At1g79630 dephosphorylates **MBP** by phosphatase assay (View interaction).
At2g30170 dephosphorylates **MBP** by phosphatase assay (View interaction).
At5g24940 dephosphorylates **MBP** by phosphatase assay (View interaction).

1. Introduction

In eukaryotes, phosphorylation and dephosphorylation of proteins are important post-translational modifications which act as a reversible switch to regulate diverse functions of proteins, such as enzyme activity, protein–protein interaction and protein localization [1]. Protein kinase mainly phosphorylates serine, threonine and tyrosine residues in eukaryotic cells [2], and protein phosphatase (PPase) removes phosphate groups from phosphorylated proteins. According to the substrate specificity, PPases are divided into two major families, serine/threonine (Ser/Thr)- and tyrosine (Tyr)-types. Ser/Thr PPases are further divided into several groups, Ser/Thr-specific PPases (PPPs) including PP1, PP2A and PP2B families, and metal ion-dependent phosphoprotein phosphatases (PPMs) including PP2C family [3,4]. The Tyr PPase family is further divided into two groups, tyrosine-specific phosphatases (PTPs), and dual-specificity phosphatases (DSPs) that catalyze dephosphorylation of both Tyr and Ser/Thr residues [5]. *Arabidopsis thaliana* has 112 PPase genes in the genome, including 23 PPPs, 69 PPMs, one PTP and 18 DSPs [6]. Compared to 90 tyrosine kinases and 105 PTP genes in human [7–9], the fact that just one PTP has been identified in Arabidopsis suggests that other non-homologous but functionally similar proteins may exist, which remain to be identified. On the contrary, very few PP2C isoforms are found in animals, while plants have many diverse PP2C families [10].

Despite the fact that there are a number of PPase genes in plants, limited reports demonstrate the role of PPases in signal transduction [11,12], possibly due to the following reasons; (1) genetic redundancy of plant PPases; (2) formation of complicated complexes required for the activation of several PPases; (3) difficulty in the expression of a large number of active recombinant PPases by traditional protein production methods, such as *Escherichia coli* cell expression system. To solve these problems, we carried out a comprehensive enzymatic assay with active recombinant proteins produced by wheat cell-free system. This plant-based protein expression system enables the production of high amounts of diverse proteins from plant and human sources, including protein kinase, ubiquitin ligase and transcription factors in an active form [13–17]. Goshima et al. (2008) reported an in vitro biochemical analysis using a large number of recombinant PPases produced by the cell-free system [18]. In this study, of the 75 synthesized human recombinant PPases, 77% of them showed PPase activity, demonstrating that wheat cell-free system is suitable for comprehensive biochemical analysis of animal PPases, and is presumably useful for plant PPases as well.

In this study, we used RIKEN Arabidopsis Full Length (RAFL) cDNA clones, which cover about 70% of Arabidopsis genes [19] as templates for protein production, and synthesized 82 recombinant Arabidopsis PPases (AtPPases). Ser/Thr and Tyr phosphatase assays were respectively conducted in vitro using Ser/Thr or Tyr phosphorylated myelin basic protein (MBP) as model substrates.

2. Materials and methods

2.1. Synthesis of streptavidin-fused AtPPase proteins

RAFL cDNA clones were used as templates for cell-free translation. DNA fragments of AtPPase, for in vitro transcription, were constructed by "split-primer" PCR as described previously [13,20]. The first round of PCR was performed using each cDNA template with 10 nM target protein specific primer (5′-CCACCCACCACCAC-CAatgnnnnnnnnnnnnnnnnn-3′; lowercase indicates the 5′-coding region of the target gene, Supplementary Table 1, column S1-primer) as the sense-primer and the AODA2306 primer (5′-AGCGTCA-GACCCCGTAGAAA) as the antisense primer. A second round of PCR was carried out using a part of the first PCR reaction mix, 100 nM Spu primer (5′-GCGTAGCATTTAGGTGACACT), 100 nM AODA2303 primer (5′-GTCAGACCCCGTAGAAAAGA) and a DNA fragment consisting of 3′-end of SP6 promoter followed by GAAΩ, streptavidin ORF, and 5′-CCACCCACCACCACCA [20]. In vitro transcription and cell-free protein synthesis were performed as described previously [21,22].

The amounts of the synthesized phosphatases were determined using ^{14}C-labeled Leu and trichloroacetic acid (TCA) precipitation as described previously [15]. The soluble fractions of the synthesized PPases were obtained by centrifugation (20000×*g* for 10 min), and the solubility was determined as the amount of the soluble fraction divided by the total amount.

2.2. In vitro phosphatase assay using ^{32}P-labeled MBP proteins

Preparation of ^{32}P-labeled MBP proteins and in vitro phosphatase assay were carried out using Protein Serine/Threonine and Tyrosine Phosphatase Assay System (Promega). MBP proteins were phosphorylated in a total volume of 200 μl consisting of 4000 U/ml Abl (protein tyrosine kinase) or 250 U/ml PKA (protein Ser/Thr kinase), 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT, 0.01% Brij 35, 3.7 mg/ml MBP, 0.25 μCi/μl [γ-^{32}P] ATP and 1 mM ATP at 30 °C for 10 h. Free [γ-^{32}P] ATP was removed by TCA precipitation, and MBP was dissolved in substrate solubilization buffer followed by dialysis at 4 °C for 16 h. The concentration of ^{32}P-labeled MBPs was determined by the Cerenkov method.

Biotin beads were suspended in 10 μl of each of the synthesized recombinant PPases for adhesion, and then washed three times with Mn^{2+} or Mg^{2+} containing protein phosphatase buffer. The PPase-coated beads were mixed with ^{32}P-labeled MBP and incubated at 30 °C for 30 min. MBP and PPase were removed by TCA precipitation, and the released ^{32}Pi was determined by the Cerenkov method.

2.3. Phosphoamino acid analysis of AtPTP1

^{32}P-Tyr-MBP was incubated with or without AtPTP1, and then separated by SDS–PAGE. MBP was extracted from poly acrylamide

gel and purified by TCA precipitation and the collected MBP was hydrolyzed by 6 N HCl at 110 °C for 1 h. After drying the products, individual amino acids were separated by 2-demensional thin-layer chromatography (TLC) using ethanol:acetic acid:water (1:1:1) (v/v) and isobutyl alcohol:formic acid:water (8:3:4) (v/v), and ^{32}P-labeled-tyrosine was detected by autoradiography.

3. Results

3.1. Comprehensive production of recombinant AtPPase

We collected the AtPPase gene information from PlantsP database (http://plantsp.genomics.purdue.edu/html/), and picked up corresponding clones from Riken Arabidopsis full-length (RAFL) cDNA clones [19]. All the AtPPase cDNA clones used in this study are listed in Supplementary Table 1. DNA templates were designed to express N-terminus streptavidin fusion PPase for high-throughput purification using biotin-coated beads, and were prepared by "split-primer PCR" [13]. Altogether 82 DNA templates, out of 95 RAFL clones (86%), were successfully synthesized.

PPases were synthesized using the wheat cell-free system [13]. To confirm the productivity and solubility of AtPPases we prepared ^{14}C-labelled PPases. The amount of the synthesized PPases in soluble fraction ranged from 0.2 to 8.9 μg/reaction (Fig. 1A), with the concentration between 0.2 and 1.8 μM (Fig. 1B). We noted that the solubility of 62 recombinant PPases was more than 50%, and 13 were completely solubilized. These results indicate that the wheat cell-free system is capable of producing many soluble recombinant AtPPases efficiently. Cold recombinant AtPPase proteins were also prepared by the same procedure for further analysis.

3.2. Phosphoamino acid analysis of tyrosine phosphatase

AtPTP1 is the only Tyr-specific PPase in Arabidopsis and its Tyr-PPase activity has been reported using recombinant protein expressed in *E. coli* [23,24]. Here we used AtPTP1 as a model AtPPase, and checked the activity of the recombinant AtPPase by phosphoamino acid analysis using ^{32}P-labeled MBP as a model substrate. As shown in Fig. 2A, 32P-Tyr-MBP was dephosphorylated in presence of AtPTP1. Phosphoamino acid analysis was carried out to confirm phospho-Tyr specific dephosphorylation on MBP by AtPTP1. ^{32}P-labeled tyrosine was detected in untreated MBP but completely disappeared in AtPTP1 treated MBP (Fig. 2B), indicating that the recombinant AtPTP1 possesses tyrosine-specific PPase activity.

3.3. Dephosphorylation activity of recombinant AtPPases

We comprehensively investigated the activity of all 82 recombinant AtPPases. The amount of free ^{32}Pi released from ^{32}P-labelled MBP was determined using liquid scintillation counter in 96-well plate format. Each streptavidin-AtPPase was bound to the surface of biotin-conjugated magnetic beads, and the beads were then mixed with either ^{32}P-Ser/Thr-MBP or ^{32}P-Tyr-MBP. It has been reported that many PPases are active in the presence of either Mg^{2+} or Mn^{2+} [18,24–37] with few exceptions, such as the human PP2Cα, that showed higher activity in the presence of Fe^{2+}, than

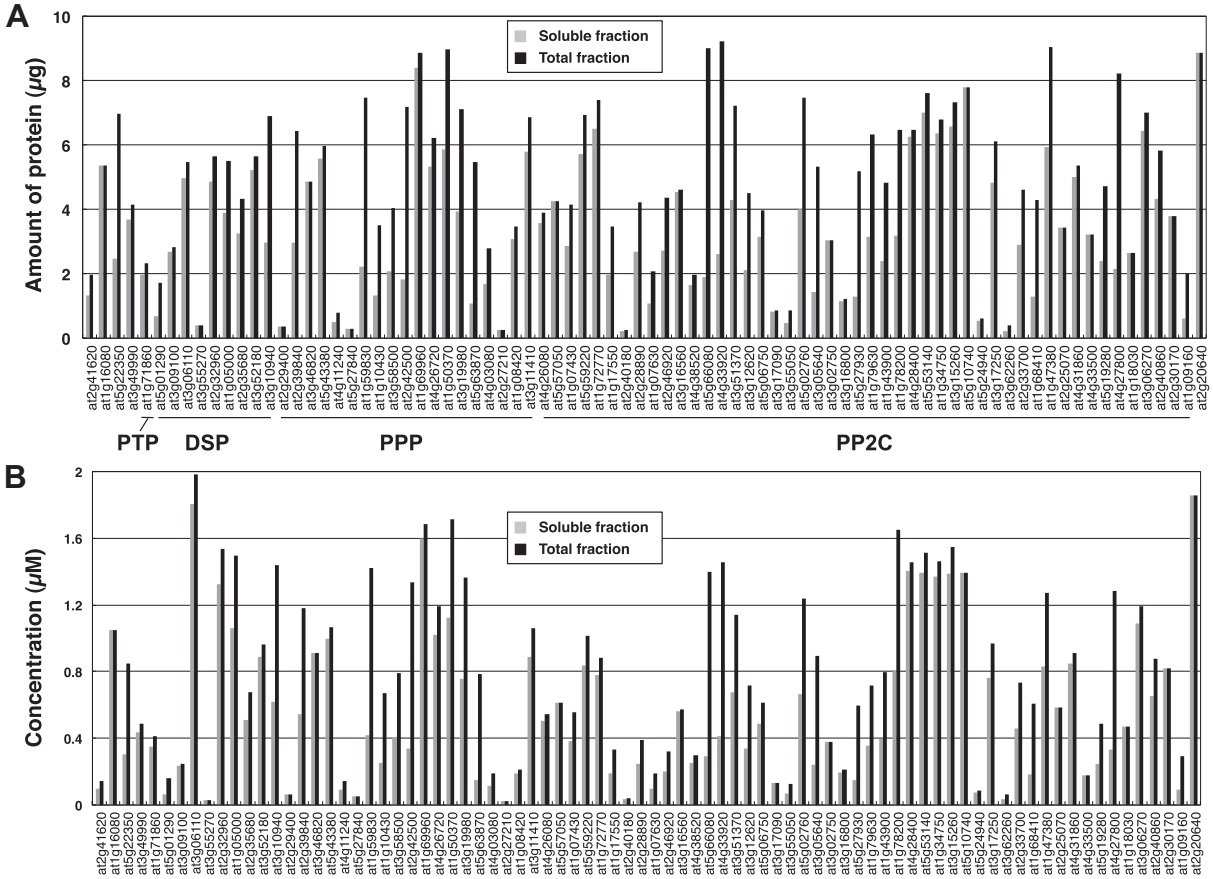

Fig. 1. Productivity and solubility of wheat cell-free expressed AtPPase. Eighty-two ^{14}C-labeled AtPPases were synthesized, and each recombinant AtPPase was applied to centrifugation and in total fraction and separated into soluble fraction and insoluble fraction. Amounts (A) and concentrations (B) of recombinant AtPPases in total fraction (black bar) and soluble fraction (gray bar) were determined by liquid scintillation method. Each PPase clone was indicated by MIPs code.

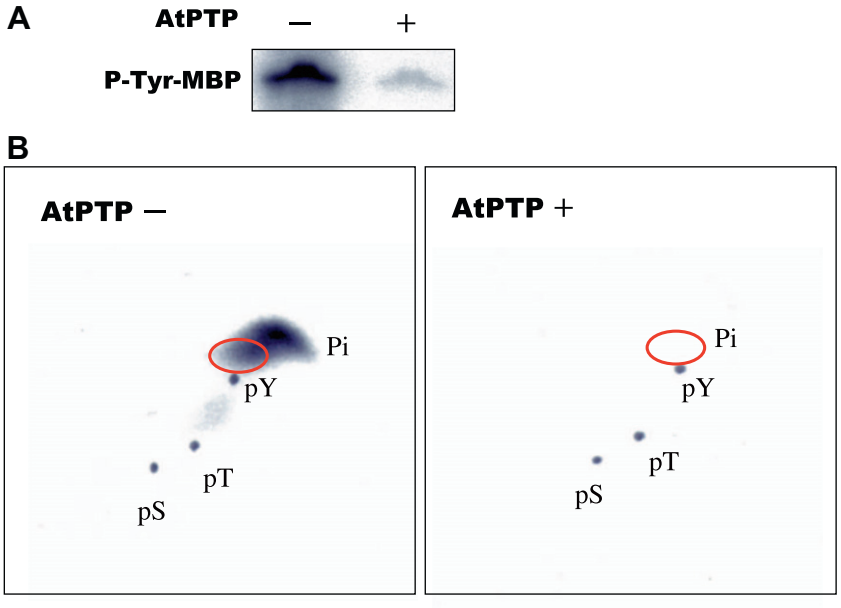

Fig. 2. Phosphoamino acid analysis of PTP1. ^{32}P-Tyr-MBP protein was incubated with or without recombinant PTP1, and then hydrolyzed by 6 N HCl. Each amino acid was separated by 2-demensional TLC, and ^{32}P-labeled tyrosine was detected by autoradiography positions of unlabeled phosphoamino standards were visualized by ninhydrin staining, and the spots were traced with autoradiography pen. The position of each standard was indicated (pS, phospho-Ser; pT, phospho-Thr; pY, phospho-Tyr; Pi, free Pi). Position of pTyr is emphasized by circle. ^{32}P-labeled tyrosine was observed only in the absence of recombinant AtPTP1.

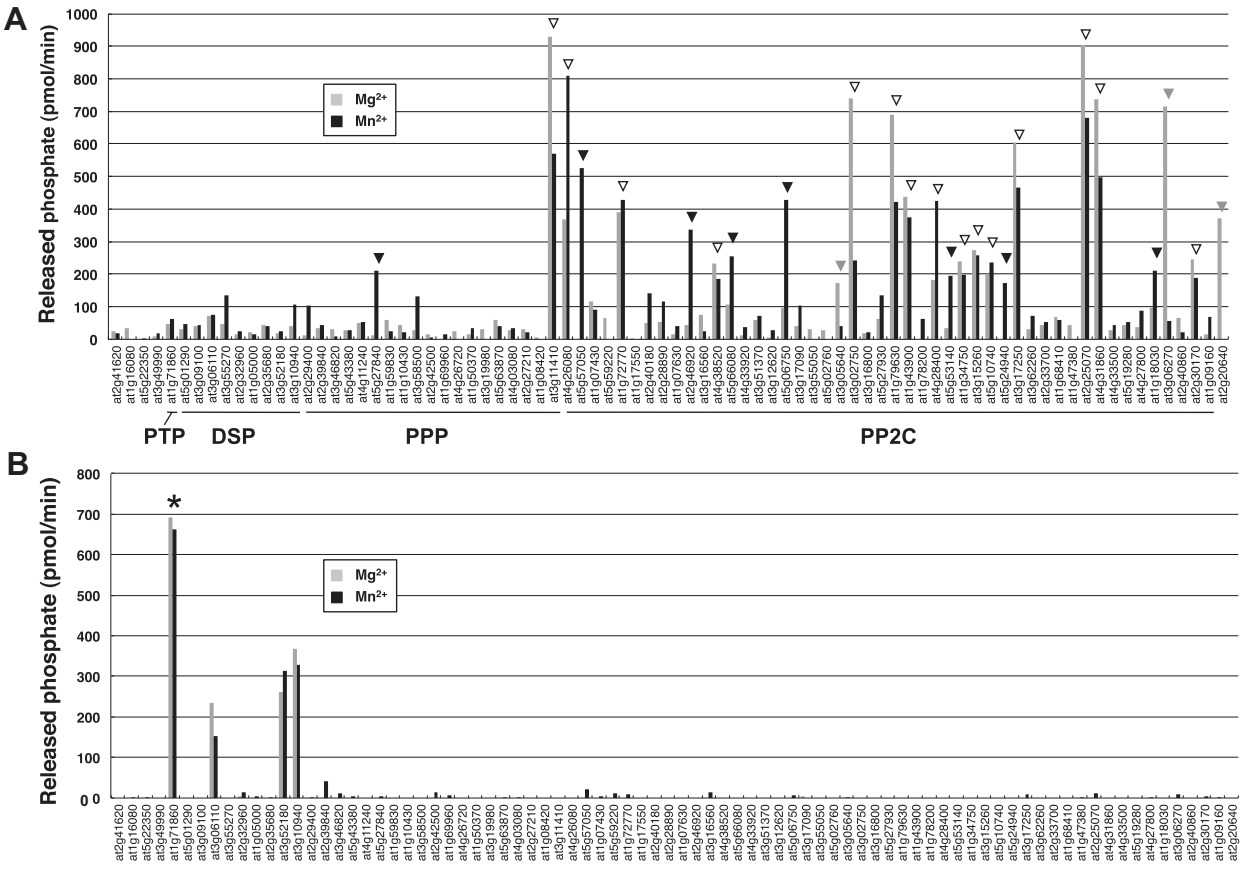

Fig. 3. Comprehensive phosphatase assay. Ser/Thr- (A) and Tyr- (B) phosphatase activity of AtPPases were measured comprehensively by using ^{32}P-Ser/Thr-MBP and ^{32}P-Tyr-MBP proteins as model substrates, respectively. The assay was carried out in presence of magnesium ion (gray bars) or manganese ion (black bars), and the result was obtained from single experiment. Asterisk indicates AtPTP1 (panel B). Opened arrowheads in panel A indicate the AtPPases that showed activity in presence of both cations. Gray and closed arrowheads respectively indicate AtPPases that showed activity with either of Mg^{2+} and Mn^{2+}.

Table 1
Summary of production and PPase activity of recombinant AtPPase in each group.

Groups	Gene no. in At genome	Tried clone no. in this study	Synthesized proteins (%[a])		Active phosphatases (%[b])			
					Ser/Thr		Tyr	
PP1	9	6	6	(100%)	1	(17%)	0	(–)
PP2A	5	5	5	(100%)	0	(–)	0	(–)
PP4	2	1	1	(100%)	0	(–)	0	(–)
PP5	1	0	0	(–)	0	(–)	0	(–)
PP6	2	2	2	(100%)	0	(–)	0	(–)
PP7	1	1	1	(100%)	0	(–)	0	(–)
PPP keich	4	3	3	(100%)	0	(–)	0	(–)
PP2C	69	49	49	(100%)	25	(51%)	0	(–)
DSP	18	9	9	(100%)	0	(–)	3	(33%)
PTP	1	1	1	(100%)	0	(–)	1	(100%)
Other Ser/Thr	2	0	0	(–)	0	(–)	0	(–)
Unknown	0	5	5	(100%)	1	(20%)	0	(–)
Total	114	82	82	(100%)	27	(33%)	4	(5%)

[a] Percentage = Synthesized protein/Tried clone × 100.
[b] Percentage = Active phosphatase/Synthesized protein × 100.

Table 2
Characterization of AtPPases exhibiting Ser/Thr- or Tyr-PPase activity in this study.

MIPs code	Other name	PlantsP number	Groups	Sub-groups in Fig. 4	Molecular weight (Da)	Additional domains	Dephosphorylated amino acids in this study	Cation required for PPase activity	PPase activity in other paper
at1g71860	AtPTP1	55804	PTP	–	37799		P-Tyr	–	[23,24]
at3g06110	AtMKP2	141908	DSP	–	18431	Potential transmembrane spanning region	P-Tyr	–	[29]
at3g52180	SEX4/PTPKIS	57127	DSP	–	39126		P-Tyr	–	[28,40]
at3g10940		36244	DSP	–	32088		P-Tyr	–	
at5g27840	TOPP8	26805	PP1	–	36039	Calcineurin-like phosphoesterase	P-Ser/Thr	Mn	
at3g11410	AHG3, AtPP2CA	26856	PP2C	A	43350	Glutamine amidotransferases class-II active site.	P-Ser/Thr	Mg, Mn	[34]
at4g26080	ABI1	26878	PP2C	A	47506	Potential transmembrane spanning region, ATP/GTP-binding site motif A (P-loop)	P-Ser/Thr	Mn	[36]
at5g57050	ABI2	26894	PP2C	A	46307	ATP/GTP-binding site motif A (P-loop)	P-Ser/Thr	Mn	[37]
at1g72770	HAB1	26832	PP2C	A	55714	Potential transmembrane spanning region	P-Ser/Thr	Mg, Mn	[34]
at2g46920	POL, POLTERGEIST	26845	PP2C	C	90363	Potential N-myristoylation site	P-Ser/Thr	Mn	[27]
at4g38520		26885	PP2C	D	43621		P-Ser/Thr	Mg, Mn	
at5g66080		26896	PP2C	D	42963	Potential transmembrane spanning region, Bipartite nuclear localization signal,	P-Ser/Thr	Mn	
at5g06750		26890	PP2C	D	43248	Potential transmembrane spanning region, Bipartite nuclear localization signal	P-Ser/Thr	Mn	
at3g05640		26858	PP2C	E	39780		P-Ser/Thr	Mg	
at3g02750		26859	PP2C	E	53715	Potential N-myristoylation site	P-Ser/Thr	Mg, Mn	
at1g79630		26824	PP2C	E	59030	Potential N-myristoylation site	P-Ser/Thr	Mg, Mn	
at3g06270		26868	PP2C	E	39260		P-Ser/Thr	Mg	
at1g43900		56278	PP2C	F	40325	Potential transmembrane helix domain, Potential transmembrane spanning region	P-Ser/Thr	Mg, Mn	
at4g28400		26880	PP2C	F	29644		P-Ser/Thr	Mg, Mn	
at5g53140		56310	PP2C	F	33582		P-Ser/Thr	Mn	
at1g34750		26823	PP2C	F	30984		P-Ser/Thr	Mg, Mn	
at3g15260		26865	PP2C	F	31630		P-Ser/Thr	Mg, Mn	
at5g10740		26891	PP2C	F	37380		P-Ser/Thr	Mg, Mn	
at5g24940		26898	PP2C	F	48120	Mitochondrial energy transfer proteins signature	P-Ser/Thr	Mn	
at3g17250		26860	PP2C	G	42256		P-Ser/Thr	Mg, Mn	
at2g25070		26851	PP2C	I	39354	Potential N-myristoylation site	P-Ser/Thr	Mg, Mn	
at4g31860		26882	PP2C	I	39203	Potential N-myristoylation site	P-Ser/Thr	Mg, Mn	
at1g18030		56026	PP2C	–	37368		P-Ser/Thr	Mn	
at2g30170		26843	PP2C	–	30895		P-Ser/Thr	Mg, Mn	
at2g20640	Pseudo gene	–	PP2C	–	31778		P-Ser/Thr	Mg	

it did with Mg^{2+} or Mn^{2+} [38]. One of our purposes in this study was evaluation of wheat germ protein expression for plant PPase functional analysis, therefore the dephosphorylation reaction was performed in the presence of Mg^{2+} or Mn^{2+}, the most general condition. The recombinant AtPTP1, whose Tyr-PPase activity had been detected by phosphoamino acid assay in Fig. 2, also showed high Tyr-PPase activity in this method (Fig. 3B, asterisk).

The PPase assay demonstrated that more than 30% of recombinant AtPPases had Ser/Thr- or Tyr-PPase activity (Fig. 3). The results are summarized in Table 1, and the PPases whose activity was observed in this study are listed in Table 2. As for PPP and PPM families, 26 PPases exhibited Ser/Thr-PPase activity (Fig. 3A). Among them, 16 of AtPPases showed Ser/Thr-PPase activity in the presence of either of Mg^{2+} and Mn^{2+} (Fig. 3A, indicated by opened arrowheads), and three and seven, only in presence of Mg^{2+} (gray arrowheads) and Mn^{2+} (closed arrowheads) respectively. Other translational mixtures showed neither Ser/Thr-PPase nor Tyr-PPase activity, indicating that there was almost no contaminated protein with PPase activity from the wheat germ extract.

More than half of the PP2C PPases were active, and a majority of them belong to PP2C subgroups A, E, F and I, [10] (Fig. 4, active PPases are shown in red characters), which have relatively smaller molecular weights and simpler structures. On the other hand, we failed to detect the activity of some of the PPases of subgroups C, D and H, which possess transmembrane domains or large extra domains (Fig. 4). None of the PPases in PP2A, PPP and PP1 groups exhibited PPase activity, although almost all the recombinant PPases in these groups had been synthesized as soluble form. One exception was TOPP8, a PP1 group PPase, which showed Ser/Thr-PPase activity. In addition to AtPTP1, three recombinant PPases, At3g06110 (AtMKP2), At3g52180 (SEX4/PTPKIS) and At3g10940, also dephosphorylated phospho-Tyr MBP in the presence of either divalent ions (Fig. 3B). Interestingly, although these three PPases belong to dual specificity phosphatase group, they did not show Ser/Thr-PPase activity (Fig. 3A, Table 1).

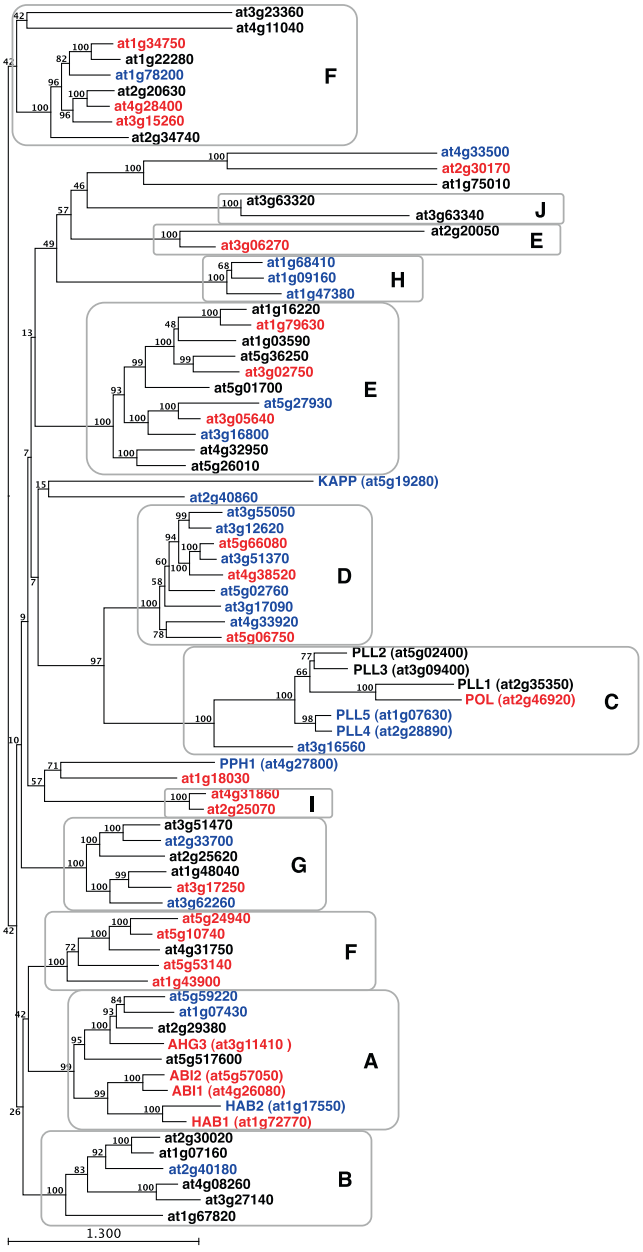

Fig. 4. Phylogenetic tree of AtPPases. Based on amino acid sequence alignment of AtPPase PP2C family, phylogenetic tree was constructed by NJ method using CLC sequence viewer (CLC bio). Red and blue characters indicate AtPPases showed activity and inactivity respectively in this study. Black characters show recombinant proteins that were not synthesized by the wheat cell-free system. The AtPPases that were not analyzed in this study were indicated by black characters. Phylogenetic subgroups (A–J) were illustrated in rectangles [10].

4. Discussion

In this study, 95 Arabidopsis PPases were selected from RAFL clones, and 82 of them were successfully synthesized in a soluble form by the wheat cell-free system. Comprehensive PPase assays of these recombinant AtPPases were carried out using ^{32}P-Ser/Thr-MBP and ^{32}P-Tyr-MBP as substrates. The results show that catalytic activity was detected in 26 Ser/Thr-PPases, three DSP-PPases and one Tyr-PPase (Fig. 2). Among them, PPase activity was observed in vitro for the first time using full-length recombinant proteins in 20 Ser/Thr-PPases and one DSP-PPase, At3g10940 (Table 2). We also detected the activity of POL, a full-length recombinant protein (at2g46920), whose activity has been confirmed by the truncated protein instead of the full-length protein [27]. These results demonstrate that the wheat cell-free system is a powerful tool for the production of active PPases.

Tyr-PPase activity of At3g10940, one of DSP-PPases, was detected in vitro for the first time. At3g10940 shows high homology with SEX4/PTPKIS (At3g52180), except that At3g10940 lacks the kinase interaction sequence domain [39,40]. DSPs are generally thought to dephosphorylate protein kinases to regulate their function [41,42]. Meanwhile, several biochemical and biological studies have suggested that AtSEX4/AtPTPKIS is a phosphoglucan phosphatase that dephosphorylates phosphate group from granules, and is related to starch metabolism in *A. thaliana* [40,43,44]. Although both AtSEX4/AtPTPKIS and At3g10940 dephosphorylated ^{32}P-Tyr-MBP in this study, it remains an intriguing issue that AtSEX4/AtPTPKIS and At3g10940 would act as PPases or phosphoglucan phosphatases in vivo.

Some AtPPases turned out to be inactive in this study, including some PPases previously reported as active (e.g. KAPP) [25]. Here we discuss several possible reasons. Firstly, the assay conditions employed, such as the pH and the cation used here and its concentration, might be unsuitable for these PPases. Optimization of assay condition is required for each inactive AtPPase. Another reason could be that some of these AtPPases might require regulatory subunits or additional factors to gain catalytic activity. Almost all PP1 and PP2A PPases were found to be inactive in this study. They generally form complexes with some regulatory subunits to maintain activity and substrate specificity [45–47]. The third reason is the substrate specificity [48–50]. In our assay, we used phosphorylated MBP as model substrate. The AtPPases that did not show the

activity might have high substrate specificity. Indeed, in this study AtMKP1, an MPK6-specific PPase [51], failed to dephosphorylate phosphorylated MBP. Lastly, some PPases might acquire activation by post-translational modifications, such as phosphorylation [45,46].

Many in vitro-synthesized recombinant AtPPases in the study displayed Ser/Thr- or Tyr-PPase activities. Although the result shown in Fig. 3 was obtained from single assay and hence we need to perform the detailed analysis to elucidate the substrate specificity and ion-requirement of each active PPase in future work, we believe that our results provided important biochemical information about their activities and biological functions that were seldom reported previously. For a majority of the other AtPPases, the amino acid sequences and gene expression profiles may offer hints about their biological functions. It is of importance to determine if these unidentified AtPPases are functional enzymes or pseudogenes, information which will be essential when they are subjected to in vivo functional analysis by reverse genetic analysis using T-DNA tag line. More recently, our group synthesized 759 Arabidopsis recombinant protein kinases using the wheat cell-free system, and 179 of these demonstrated autophosphorylation activity [17]. Biochemical studies using sets of recombinant protein kinases and PPases will provide rich information about protein phosphorylation/dephosphorylation pathways in plant.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.08.014.

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